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(54) IMMUNOTOLERANCE INDUCTION AGENT

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain an immunotolerance induction agent capable of maintaining transplanted organs without necessitating the maintenance by means of immunosuppressants.
SOLUTION: This immunotolerance induction agent is a medicine used for induction of immunotolerance and is composed of the first medicinal composition for portal vein administration which includes toletogens containing stem cells, hematopoietic prodromal cells, mature lymphocytes or these mixtures as an active ingredient and the second medicinal composition for intravenous administration containing tolerogens as an active ingredient.

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NOTICES *

CLAIMS

Claim(s)]
Claim 1] The immunological tolerance inducer characterized by consisting of the 2nd physic constituent for ntravenous administration which makes an active principle the 1st physic constituent and the above-mentioned olerogen for the administration in a portal vein which are the physic used for induction of immunological olerance, and make an active principle tolerogen containing a hematopoietic stem cell, a hemopoietic precursor ell, a mature lymphocyte, or these mixture.
Claim 2] The immunological tolerance inducer according to claim 1 which makes a bone marrow cell tolerogen.

Translation done.]

NOTICES *

)ETAILED DESCRIPTION

Detailed Description of the Invention]
)001]
Field of the Invention] This invention relates to the immunological tolerance inducer which can attain an organ ransplantation and the immunological tolerance which enables maintenance of a transplant in more detail.
)002]
Description of the Prior Art] For the organ transplantation, the immunosuppresant serves as an indispensable xistence and a new immunosuppresant is developed now one after another. This immunosuppresant is divided ıto two from the purpose of use (application). One is the purpose which controls rejection preclusively, as long s a transplant is in the inside of the body every day, it continues being taken, and it is called a maintenance ımmunosuppresant, a prophylactic immunosuppresant, a fundamental immunosuppresant, etc. Another side is the ejection whose symptoms are shown in spite of carrying out maintenance immunosuppression, and the purpose /hich mainly treats cellularity rejection, although it is short period of times, is used for performing ımmunosuppression in large quantities and powerfully, and is called a rejection therapy agent etc.
0003] however, immunosuppresants, such as this, -- both the principal action and a side effect -- although -- ince it cannot say that it is harmless for the body but maintenance, extensive administration, etc. by the hronic administration are needed, it is accompanied by the remarkable toxicity thru/or the remarkable side ffect that neither can be disregarded. Moreover, the rejection which could not do so immunosuppression ffectiveness sufficient in independent administration of an immunosuppresant, and showed the symptoms of it annot be treated to high rate.
0004] On the other hand, clinically, in spite of having not made administration of an immunosuppresant, the eport by which a transplant is maintained safely has appeared here and there, and since the immunological olerance condition was guided, this is considered. According to formation of this immunological tolerance, ıdministration of the above-mentioned immunosuppresant becoming unnecessary, therefore guiding ımmunological tolerance artificially attracts attention as a policy objective in an organ transplantation, and arious research results are reported about this.
0005] As this artificial tolerance induction approach, the following reports are referred to, for example.
0006] Tolerance induction by concomitant use with tolerogen (tolerogen) import and the antimitotic agent antimitoticdrug) of a spleen cell or a bone marrow cell [Fukuoka acta Med., 81 (1), 20-40; (1990) Microbiol.Immunol., 32 (3), 283-292 (1988), etc.]. As an antimitotic agent here 6-mercaptopurine (6-nercaptopurine), Methotrexate (methotrexate), SAIKURO phosphaMAIDO (cyclophosphamide, CP), 5-fluorouracil 5-fluorouracil), azathioprine (azathioprine, AZP), procarbazine (procarbazine), etc. are mentioned. Since the nechanism of action is notably different from antimitotic agents, such as this, Cyclosporin A (ciclosporin A, CsA) ınd the steroids shall not be suitable for induction of immunological tolerance.
0007] Hayakawa and others -- FK506 -- using -- a donor -- [the Keio medicine, 72 (3), and 163-176] which ıave reported the attempt which guides a specific immunosuppression condition (1995). Similarly, Muramatsu ınd others has reported the possibility of the tolerogenesis by 15-DSG [the 20th Japanese Society of Reconstructive Microsurgery abstracts and 89 - 90 pages (1994)].
0008]
Problem(s) to be Solved by the Invention] The purpose of this invention is to offer the technique of making lesired immunological tolerance attaining in an organ transplantation (you making it materialized). That is, it is in offering the new technique which can avoid certainly the serious side effect which does not need the naintenance (chronic administration of an immunosuppresant) by the current immunosuppresant, therefore is

ollowed on this, and enables maintenance of a transplant.
0009] this invention persons came to complete this invention for the drugs of the following configuration greeing for the above-mentioned purpose a header and here wholeheartedly as a result of research.
0010]
Means for Solving the Problem] It is the physic which is used for induction of immunological tolerance according o this invention, and the immunological tolerance inducer characterized by consisting of the 2nd physic constituent for intravenous administration which makes an active principle the 1st physic constituent and the above-mentioned tolerogen for the administration in a portal vein which make an active principle tolerogen containing a hematopoietic stem cell, a hemopoietic precursor cell, a mature lymphocyte, or these mixture, especially the above-mentioned immunological tolerance inducer which makes a bone marrow cell tolerogen are offered.
0011] According to use of this invention inducer, the immunological tolerance of the request corresponding to he purpose mentioned above can be attained, and good maintenance of the transplant in an organ ransplantation is attained.
0012]
Embodiment of the Invention] this invention immunological tolerance inducer makes indispensable both 1st physic constituent for the administration in a portal vein, and 2nd physic constituent for intravenous administration as above-mentioned, and there is especially no limit in the physic gestalt of these constituents, or he gestalt with which use is presented in the limitation.
0013] For example, the above 1st and the 2nd physic constituent can be included in one physic gestalt, or can lso divide them as a special physic gestalt by request. That is, as long as the effectiveness of this invention of ttaining desired immunological tolerance is done so so that it may be represented by the example of use of this nvention physic mentioned later, there is especially no limit in the physic gestalt or the gestalt with which use is resented.
0014] As tolerogen containing the hematopoietic stem cell and hemopoietic precursor cell which are the active rinciple which is common in the 1st and 2nd physic pharmaceutical preparation concerning this invention, a nature lymphocyte, or these mixture, the thing of the transplant donor (with mouse, it is donor and affiliated) rigin can be mentioned, for example, and this can be the bone marrow cell containing these cells, a spleen cell, eripheral blood cells, or those mixture.
0015] Separation and isolation of tolerogens, such as this, can follow a well-known approach. For example, this pproach in a mouse is "cell immunity experiment operation information" [Mishell B.B. and Shgi S.M. It is lescribed by editing, Katsuyuki Imai, Kawaguchi **** Harada [Takayuki] ****, Riko-Gaku-Sha, 3 - 12 pages, nd 1982]. That is, after slaughtering a mouse under anesthesia and disinfecting a body surface using ethanol 0%, a right-hand side antinode is cut open, a spleen is extracted in sterile, this is unfolded with non-** incettes on the stainless steel mesh of 200 gages in RPMI1640 solution (Nikken Bio Med.Lab.), and it considers s isolation spleen cell suspension. After RPMI1640 solution washes a isolation spleen cell once, it hemolyzes with a tris hydrochloric-acid ammonium solution (0.75% NH4Cl, 0.017 M Tris-HCl, pH 7.5), and after RPMI1640 olution washes twice [further], it prepares in this solution. Moreover, extraction of a bone marrow cell and reparation slaughter a mouse under anesthesia. The skin of a biped is stripped after disinfecting a body surface sing ethanol 70%. After dissociating from the truncus, with muscles attached and cutting off near muscles with cissors, The joint capsule and muscles are completely removed with sterile absorbent gauze. To each of a emur and a tibia 22 gage needles (Code No.NN-2225R, Terumo Co., Ltd) attached to the syringe (2.5ml, Code No.SS-02S, Terumo Co., Ltd) from the knee-joint side are stabbed. A bone marrow cell is washed away with RPMI1640 solution in a syringe to a sterilization petri dish (90x15mm, Iwaki Clinical Test Ware), and it can carry ut by preparing in RPMI1640 solution.
0016] As tolerogen from a transplant donor (Homo sapiens), use of a bone marrow cell and a peripheral blood ell can be illustrated suitably, and acquisition of these cells is common knowledge at this contractor. For example, on the occasion of use of a bone marrow cell, case [in a bone marrow transplantation], it can apply.
0017] The use of tolerogen which consists of the bone marrow cell containing many hemopoietic precursor ells, the spleen cell containing a mature lymphocyte (except for an activated lymphocyte), these peripheral lood cells, or those mixture as the above-mentioned 1st physic pharmaceutical preparation is desirable, and use f the tolerogen which consists of the above-mentioned bone marrow cell as the 2nd physic pharmaceutical reparation on the other hand is desirable. In addition, acquisition of a cell can illustrate more nearly similarly than an easy point the peripheral blood cell which contains the hematopoietic stem cell mobilized from bone

narrow, for example by cytokine, such as G-CSF, as an active principle in both of the above-mentioned 1st and nd physic pharmaceutical preparation although use of a bone marrow cell is desirable as a desirable active rinciple from a mature lymphocyte and the both element of a hemopoietic precursor cell being included again.
0018] In the 1st and 2nd physic pharmaceutical preparation, this active principle can be prepared to general hysic formulation like the various physic pharmaceutical preparation which usually consists of this kind of cell omponent. As this physic pharmaceutical preparation, various kinds of gestalten can choose by request, and jections can be illustrated as the typical thing. What is known better [in this field] also as support of the arious kinds permitted pharmacologically used on the occasion of preparation to physic formulation, such as his, than before can be used widely, and that preparation can also follow a conventional method. Use of various inds of pharmaceutical preparation for infusion solutions by which the current general purpose is carried out on he occasion of preparation of pharmaceutical preparation, such as this, is also possible.
0019] In addition, in this invention, the above-mentioned physic pharmaceutical preparation can also be repared on the occasion of transplantation at the time of the object for transplant donor twists.
0020] In the 1st and 2nd physic pharmaceutical preparation in this invention, the 1st physic pharmaceutical reparation makes administration in a portal vein indispensable, and the 2nd physic pharmaceutical preparation nakes intravenous administration indispensable. If the mode of typical administration is illustrated, the 1st physic harmaceutical preparation will be first prescribed for the patient in a portal vein, and the 2nd physic harmaceutical preparation will be administered intravenously after that. After the 1st administration in a portal ein, this 2nd intravenous administration is good to be carried out at the stage (a mouse the 5th day) which was oing up again, after the reactivity over the alloantigen of the donor of a host cell serves as min in the mixed ymphocyte reaction of splenic cells (for example, a mouse the 4th [about] day).
0021] In the stage when reactivity [as opposed to / in the dose in a portal vein of the 1st physic harmaceutical preparation / a donor's alloantigen by the mixed lymphocyte reaction after the administration in portal vein] serves as min (reaction control is max) It is appropriate to make the minimum dosage (a mouse x107 pieces) for whenever [reaction control] to arrive at a plateau into a standard. Moreover, the amount of ntravenous administration of the 2nd physic pharmaceutical preparation In the bone marrow transplantation (a nouse after the radiation irradiation of a lethal dose) of the usual major histocompatibility complex (MHC) onconformance, sufficient amount (a mouse 3x107 pieces) to reconstruct a host's immune system can be made nto a standard.
0022] In addition, in the above, the mixed lymphocyte reaction trial of splenic cells can be carried out according o a conventional method, and the inside of the portal vein in [the above "cell immunity experiment operation nformation", 147 - 149 pages and 1982] and Homo sapiens and the amount of intravenous administration can pply to it in the usual bone marrow transplantation. For example, the dose beyond about 3x108 pieces/kg or it an be illustrated as a bone marrow cell.
0023] By treatment which consists of administration from the vein of the 2nd physic pharmaceutical preparation f this invention which follows administration from the portal vein of the 1st physic pharmaceutical preparation f this invention in this way, desired immunological tolerance is guided and good maintenance of a transplant is ttained.
0024] According to the treatment of this invention, desired immunological tolerance is guided, good maintenance f a transplant is attained, but this shows effectiveness regardless of the stage of transplantation enforcement . f a transplant. Therefore, the transplantation concerned can be performed to all after the immunological olerance by this invention treatment was attained in parallel with this invention treatment good.
0025] In addition, unless the effectiveness of this invention is injured on the occasion of induction of the mmunological tolerance concerning this invention, various kinds of medical aid by which usually being used on he occasion of this kind of treatment is known, administration of other physic pharmaceutical preparation, etc. an also be used together. 1 of for example, concomitant use of various kinds of immunosuppresants currently sed for the induction of immunological tolerance mentioned above as the example, 2nd day extent after the dministration in a portal vein, and 5th day extent especially short-term [after the administration in a portal vein f the 1st physic pharmaceutical preparation] thru/or two concomitant use of an immunosuppresant can be llustrated. Here, as an immunosuppresant which can be used, typically, Cyclosporin A and FK506 grade can be llustrated and the amount of concomitant use, direction for use, etc. can follow them of a known commercial tem.
0026]
Example] Hereafter, in order to explain this invention in more detail, the example of a trial performed by being

ıttached to this invention active principle is given.
0027]
The example 1 of a trial] The injection in a portal vein of (1) different ** donor's splenic cells or a bone marrow :ell and the intravenous injection of (2) different ** donor's bone marrow cell performed tolerance induction in he following examples of a trial, and immunological tolerance formation observed extent of the take by ransplantation of the skin (a donor and affiliated) which is the organ which is the easiest to receive rejection, ınd was taken as the index.
0028] (1) From the 8 weeks old feminity BALB/cCrSlc mouse (19 to 22 g weight, and BALB/c; Japan SLCInc.) ıf preparation of splenic-cells suspension, splenic cells were extracted, it unfolded with the non-** pincettes on he stainless steel mesh of 200 gages in the RPMI1640 solution (Nikken Bio Med.Lab.), and the isolation spleen :ell was obtained. After it hemolyzed after 1-time washing in RPMI1640 solution with the tris hydrochloric-acid ımmonium solution (0.75% NH4Cl, 0.017 M Tris-HCl, pH 7.5) and RPMI1640 solution washed this twice [further], ı this liquid, splenic cells were made to float and splenic-cells suspension (1.5x108/ml concentration) was ırepared.
0029] (2) Remove a femur and a tibia from the 8 weeks old feminity BALB/c mouse of preparation of bone narrow cell suspension. 22 gage needles (Code No.NN-2225R, Terumo Co., Ltd.) attached to the syringe (2.5ml, Code No.SS-02S, Terumo Co., Ltd.) from the knee-joint side are stabbed, respectively. RPMI1640 solution in a :yringe -- a bone marrow cell -- a sterilization petri dish (90x15mm, Iwaki ClinicalTest Ware) -- a push style -- he back the bottom Made it suspend in RPMI1640 solution, the bone marrow cell obtained was made to float ıfter 1-time washing and in this solution with RPMI1640 solution, and desired bone marrow cell suspension 1.5x108/ml concentration) was prepared.
0030] (3) Shave and disinfect 10 weeks old feminity C57BL of injection in a portal vein / 6CrSlc mouse (20 to !4 g weight, and B6; Japan SLCInc.) under pentobarbital (Pitman-Moor Inc.; 37.5 mg/kg weight i.p.) anesthesia. \fter performing abdomen median incision, expose a mesentery and 27 gage needles (Terumo Co., Ltd.) attached o the syringe for 1ml-tuberculin are made to stab through mesentery fat tissue. Injection administration of !x107 (suspension 0.2ml) of the splenic cells of the BALB/c mouse prepared above (1) or a bone marrow cell vas carried out into the portal vein.
0031] (4) The bone marrow cell suspension obtained with the intravenous injection above (1) was adjusted to x108/ml concentration, and injection administration of the 3x107 pieces (0.3ml) was carried out on the 5th the njection in a portal vein of the above (3) back [caudal vein / of a host mouse].
0032] (5) The skin graft was performed after the injection in a skin-graft portal vein on the 7th. Adjustment and he transplantation approach of a skin graft were performed with reference to the approach [Mayumi et al., Jpn.J.Surg., 18, and 548-557 (1988)] given in reference as follows.
0033] That is, it was slaughtered under ethyl ether (NacalaiTesque Inc.) anesthesia, having used the 8-weeks ıld BALB/c mouse as the donor. The hair of the whole body was removed in the depilating agent (Feather Hair Remover, Feather Safty Razor Co., Ltd.), and after disinfecting with an alcoholic solution 70%, exfoliation :xtraction of all the skin layers was carried out. After exfoliating a subcutaneous adipose tissue as much as ıossible using a pincette (point deflection taper non-**) and a sterilization cotton swab, the fragment was :arried out to the piece of the skin (1.2x1.5cm around), and it was made to float in the sterile phosphate-ıuffered saline (Dulbecco's PBS (-), Nissui Pharmaceutical Co., Ltd.) which added 1mm incision to one side by :he side of the head as a marker, and was cooled.
0034] After anesthetizing B6 host mouse by pentobarbital (37.5 mg/kg weight i.p.), it carried out depilating of :he right back (3.0x3.5cm around) by the epilation with a finger, and said depilating agent, it disinfected with the ılcoholic solution 70%, and the field of operation for transplantation was produced.
0035] The marker was turned to the tail, the piece of the skin of BALB/c which carried out [above-nentioned] preparation was installed in the stripped plane, and eight stitches (the center of four sides and four ıngles) were sutured with the nylon suture with 6 -zero stitch (Ethilon; Ethicon Inc.). The skin-graft side was :overed with gauze with fradiomycin sulfate ointment (2.0x2.5cm around, Sofratulle; Japan Roussel Co., Ltd.), ınd it wound with adhesive flexible dressings (Elatex; Alcare Co., Lrd.) further.
0036] The existence of skin-graft take performed observation [week / 2nd] after transplantation.
0037] (6) A result result is shown in the following table 1.
0038]
Table 1]

	寛容処置		皮膚移植生着	
	p.v. 投与	i.v. 投与	移植後経過時間(週)	生着率 (%)
試験群1	脾細胞	骨髄細胞	36	100 (10/10)
試験群2	脾細胞	脾細胞	18	20 (1/5)
試験群3	骨髄細胞	骨髄細胞	36	67 (4/6)
対照群1	脾細胞	−	3	0 (0/4)
対照群2	骨髄細胞	−	3	0 (0/4)

0039] (7) As a result of carrying out the intravenous injection of the bone marrow cell of a BALB/c mouse on he 5th after MHC of ten animals injecting with explanation of a result, and the splenic cells of a consideration rial group 1:BALB/c mouse in the portal vein of incongruent B6 mouse and performing the skin graft on the 7th, he skin graft carried out take with ten mice among ten animals after transplantation at the time for the 36th veek.

0040] Trial Group 2: Although the transplant carried out take with one mouse among five animals after ransplantation at the time for the 18th week as a result of carrying out the intravenous injection of the splenic ells of a BALB/c mouse on the 5th after injecting with the splenic cells of a BALB/c mouse in the portal vein f five B6 mice and performing the skin graft on the 7th, the skin graft was refused in the 6th week at one nimal, and was refused with three mice in the 7th week (it dropped out).

0041] Trial Group 3: As a result of carrying out the intravenous injection of the bone marrow cell of a BALB/c nouse on the 5th after injecting with the bone marrow cell of a BALB/c mouse in the portal vein of six B6 mice nd performing the skin graft on the 7th, the skin graft carried out take with four mice among six animals after ransplantation at the time for the 36th week.

0042] Control-group 1: After injecting with the splenic cells of a BALB/c mouse in the portal vein of four B6 nice, as a result of performing the skin graft on the 7th, it is two mice in the 2nd week after transplantation, and he skin graft was refused with the two remaining mice in the 3rd week (it dropped out).

0043] Control-group 2: After injecting with the bone marrow cell of a BALB/c mouse in the portal vein of four 36 mice, as a result of performing the skin graft on the 7th, it is two mice in the 2nd week after transplantation, nd the skin graft was refused with the two remaining mice in the 3rd week.

0044] According to intravenous administration of the 2nd physic pharmaceutical preparation after the dministration in a portal vein of the 1st physic pharmaceutical preparation, the above result shows that the take maintenance of immunological tolerance specific to a donor's alloantigen) of the donor skin graft over a long eriod of time can carry out.

0045] In addition, in the above-mentioned trial group 3, when prescribing the immunosuppresant for the patient etween the administration in a portal vein of a bone marrow cell (the 0th day), and intravenous administration the 5th day), improvement in the rate of take of a skin graft was accepted. Hereafter, the example of a trial vhich clarifies this is given.

0046]

The example 2 of a trial]

1) It was made to be the same as that of (2) of the inside of a portal vein, and the example 1 of the intravenous njection above-mentioned trial, (3), and (4) in the preparation list of a bone marrow cell.

0047] (2) Intraperitoneal injection of cyclosporin A(Ciclosporin A, Cs A: Sandimmun, 250mg / 5ml solution, and Sandoz) 10 mg/kg weight or the FK506(10mg / 1ml solution, Fujisawa Pharmaceutical Co., Ltd.) 1 mg/kg weight vas carried out with the 2nd day after the injection in a portal vein as an administration immunosuppresant of an mmunosuppresant on the 5th.

0048] (3) It was presupposed that it is the same as that of (5) of the example 1 of a skin-graft trial.

0049] (4) A result result is shown in the following table 2.

0050]

Table 2]

	寛容処置			皮膚移植生着	
	p.v.投与	免疫抑制剤投与	i.v.投与	移植後経過時間(週)	生着率(%)
試験群3	骨髄細胞	−	骨髄細胞	36	67(4／6)
試験群4	骨髄細胞	CsA	骨髄細胞	32	80(4／5)
試験群5	骨髄細胞	FK506	骨髄細胞	30	83(5／6)
対照群2	骨髄細胞	−	−	3	0(0／4)

0051] (5) It is as having explained explanation, the consideration trial group 3, and control group 2 of a result in ull detail for the example 1 of a trial.
0052] Trial Group 4: After injecting with the bone marrow cell of a BALB/c mouse in the portal vein of five B6 nice, Although the skin graft was refused with one mouse in the 6th week after transplantation as a result of prescribing CsA for the patient with the 2nd day on the 5th, and also carrying out the intravenous injection of he bone marrow cell of a BALB/c mouse on the 5th and performing the skin graft on the 7th, the skin graft arried out take with four mice among five animals after transplantation at the time for the 32nd week.
0053] Trial Group 5: After injecting with the bone marrow cell of a BALB/c mouse in the portal vein of six B6 nice, Although the skin graft was refused with one mouse in the 6th week after transplantation as a result of prescribing FK506 for the patient with the 2nd day on the 5th, and also carrying out the intravenous injection of he bone marrow cell of a BALB/c mouse on the 5th and performing the skin graft on the 7th, the skin graft arried out take with five mice among six animals after transplantation at the time for the 30th week.
0054] Things, such as this, show the following thing. That is, although it should not have been suitable for the mmunosuppresant of CsA or FK506 grade using together with tolerogen, and making immunological tolerance uide conventionally, when using this together about the combination of the administration in a portal vein of the st physic pharmaceutical preparation as an immunity inducer according to this invention, and intravenous administration of the 2nd physic pharmaceutical preparation, improvement in the rate of skin take was accepted, nd it became clear that it is effective also in immunological tolerance.
0055] According to this invention, clinical application can offer a fully expectable new tolerance induction echnique as mentioned above.
0056]
The example 3 of a trial]
1) It was made to be the same as that of (2) of the inside of a portal vein, and the example 1 of the intravenous njection above-mentioned trial, (3), and (4) in the preparation list of a bone marrow cell.
0057] (2) Intraperitoneal injection of the 10 mg/kg weight of the administration CsA of an immunosuppresant vas carried out with the 2nd day after the injection in a portal vein on the 5th.
0058] (3) It carried out like (5) of the above-mentioned example 1 of a trial except having carried out the skin graft on the injection in a skin-graft portal vein, and the same day (n= 6). In addition, the group which used the piece of the C3H mouse skin as contrast was set (n= 4).
0059] (4) A result result is shown in drawing 1 .
0060] this drawing -- setting -- an axis of ordinate -- the rate of skin-graft take (%) -- in an axis of abscissa, a group 1 shows a trial group and a group 2 shows a control group for the elapsed time (week) from the skin graft, respectively.
0061] By the result of this example of a trial, it became clear that it is possible to perform the actuation and he organ transplantation of tolerance induction by the administration in a portal vein of the 1st physic pharmaceutical preparation to coincidence. So, in the case of Homo sapiens, operation of the administration in a portal vein and the organ transplantation of the 1st physic pharmaceutical preparation (bone marrow cell etc.) rom a brain-dead donor is attained at coincidence. Even if this approach does not remove the T cell in bone narrow, it does not show the symptoms of a graft versus host reaction (GvHR), either, but the immunological olerance prolonged by two administration also of an immunosuppresant becomes possible, and it considers it to be an epoch-making approach.
0062]
The example 1 of pharmaceutical preparation] A bone marrow cell or splenic cells is suspended in a

hysiological saline, and 1x108 cells / pharmaceutical preparation for portal vein administration of ml is prepared.
)n the other hand, the pharmaceutical preparation for vein administration of bone marrow cell 1x108 cell / ml
hysiological saline is prepared similarly.
0063] As for the above-mentioned pharmaceutical preparation for portal vein administration, in the case of
lomo sapiens, it is desirable that a medicine is usually prescribed for the patient with the bone marrow cell
good even if T cell is mixing) dose 3x108 cell / more than kg.

Translation done.]

< NOTICES *

.This document has been translated by computer. So the translation may not reflect the original precisely.
.**** shows the word which can not be translated.
.In the drawings, any words are not translated.

)ESCRIPTION OF DRAWINGS

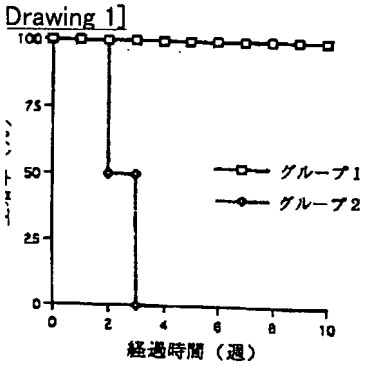
Brief Description of the Drawings]
Drawing 1] It is the drawing in which the result of the skin-graft take in the example 3 of a trial is shown.

Translation done.]

: NOTICES *

)RAWINGS

Drawing 1]



Translation done.]